PATENT
514413-3550.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Wolf-Bernd Frommer
Serial No.	:	09/854,774
Filed	:	May 14, 2001
For	:	DNA SEQUENCES FOR AN AMINO ACID TRANSPORTER, PLASMIDS, BACTERIA, YEASTS AND PLANTS CONTAINING A TRANSPORTER AND THEIR USE
Examiner	:	R. Kallis
Group Art Unit	:	1638

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AF Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Marilyn Matthes Brogan, Reg. No. 31,223

Name of Applicant, Assignee or Registered Representative

Signature

February 12, 2004

Date of Signature

DECLARATION UNDER 37 C.F.R. 1.132

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Marion Kwart, declare and state that:

1. My *curriculum vitae*, demonstrating my education, training and experience is appended hereto. I am familiar with the applications and the prosecution history of the patent and application (U.S. application Serial No. 08/362,512, its granted patents and divisional application Serial No. 09/854,777). I am considered by my peers to be an expert in the field to which the application pertains, and I am otherwise qualified to speak and render expert opinions as to the present application and its invention.

2. Claims 21-28 and 31-83 of the instant application have been rejected under 35 U.S.C §112, first paragraph, as allegedly being non-enabled. I respectfully disagree with the

Examiner, and state that the specification provides sufficient guidance to a person skilled in the art to perform the invention in other plant species, apart from *Arabidopsis thaliana*, which was used as a model plant (and working example) in the present application.

Specifically, pages 4 and 5 of the specification provide a process for the identification and isolation of DNA sequences from plants that encode amino acid transporter proteins. In fact, this process was specifically designed by the inventor, Wolf-Bernd Frommer, in order to allow the testing of basically any cDNA library from any plant with the help of a (double) mutant yeast strain, and this process forms a part of the presently claimed invention.

The cloning, characterization and transformation of plants with the isolated plant amino acid DNA is described in the subsequent examples. Further details of the mutant yeast strain to be used are provided on page 20, lines 9-12 of the specification. The corresponding yeast strain is available to the public, and it is immediately clear to the skilled artisan from the disclosure of the present application how to use the mutant yeast stain in the identification and isolation process, as described on pages 4 and 5 of the specification, in connection with the working examples. Thus, it is submitted that the specification is enabling to a person skilled in the art and requires no undue experimentation on the part of the skilled person to follow the methods of the invention.

Additionally, I assert that a skilled artisan, reading the instant specification, would know how to identify plant amino acid transporter genes and express them in transgenic plants.

Due to the nature of the "mutant yeast test system", its application is not restricted to any specific plant cDNA library. Rather, the same mutant yeast strain may be used for any available plant cDNA of interest to be introduced into the yeast. Any additional information the skilled artisan might need in order to use the claimed methods for the identification of DNA sequences encoding plant amino acid transporters are also provided in the present application. Specifically, pages 23 to 27 of the specification provide sufficient details on the methods and materials to be used. These assertions are supported by the results of several studies, which are discussed below and in the accompanying Amendment.

3. While the cloning of bacterial and yeast genes encoding amino acid transport proteins, and the characterization of microbial mutants deficient in amino acid uptake, revealed the presence of a whole set of transport proteins in bacteria and yeast, plant studies did not show any individual membrane transport activity. The availability of microbial mutants allowed the

detection and characterization of individual microbial transport proteins responsible for certain chemical groups of amino acids or even for individual amino acids (Tanaka and Fink, 1985, Gene 38: 205-214; Vandenbol *et al.*, 1989, Gene 83: 153-159; Wallace *et al.*, 1990, J. Bacteriol. 172: 3214-3220). However, uptake kinetic studies in plants yielded complex data because whole suspension cells or plasma membrane vesicles were used to study plant amino acid transport. Although Li and Bush (1990, Plant Physiol. 94: 268-277) succeeded to distinguish four groups of transport systems, no further resolution to individual transport activities could be obtained. In fact, until 1993, neither a plant membrane protein, nor its corresponding gene responsible for amino acids transport, was identified. Knowing that the allocation of amino acids within the plant is essential for growth and yield, there was a great need in the art to identify such transport systems and to characterize amino acid transport processes in more detail.

The present invention filled that need by providing artisans in the field with an invaluable research tool in the form of the first cDNA encoding a plant amino acid transport protein. The invention proved the yeast complementation system to be the most efficient tool to identify heterologous genes from different species, including plants. Further, this heterologous complementation of yeast mutants allowed researches not only to prove functionality, but also to characterize the biochemical properties of the isolated plant transport protein.

Since the time of the invention, other amino acid transporters from plants different from Arabidopsis have been characterized using the same or other yeast mutants as complementation systems: Ricinus (Marvier *et al.*, 1998, Biochim. et Biophys. Acts, 1373: 321-331); tomato (Schwacke *et al.*, 1999, The Plant Cell 11: 377-391); broad bean (Montamat *et al.*, 1999, Plant Mol. Biol. 41 (2): 259-268); pea (Tegeder *et al.*, 2000, Plant Phys. 122 (2): 319-325); and common ice plant (Popova *et al.*, 2003, Plant Mol. Biol. 52 (3): 569-578).

The use of different yeast mutants deficient in specific amino acid transport proteins enabled the isolation of amino acid transporters showing new sequence homology and different transport properties. Depending on the sequence homologies, whole families of amino acid transporters could be divided into the AAP-family and the ProT-family (Rentsch *et al.*, 1996, Plant Cell 8: 1437-1446).

Furthermore, yeast complementation has enabled the isolation of a gene families encoding peptide transport proteins in plants: the PTR-family (Steiner *et al.*, 1994, Plant Cell 6: 1289-1299), and the NTR-family (Rentsch *et al.*, 1995, FEBS 370: 264-268; Rentsch *et al.*,

1996, Plant Cell 8: 1437-1446). In addition, the use of the "double mutant yeast strains" has proven to be an invaluable tool to identification of other transporters in plants, such as oligosaccharide transporters (WO 94/00574, US 5,608,146 and US 5,981,181) and ammonium transporters (US 6,620,610 and US 08/635,967). All of these plant membrane transport proteins were not only isolated via yeast complementation. Each of them have been proved for functionality and have been characterized biochemically in detail.

Moreover, "double mutant yeast strains", as described in U.S. application Serial Nos. 08/362,572; 08/964,939 and 09/854,774 may be used in the isolation of insertional mutants that are defective in the expression of amino acid transporters and to identify DNA sequences suitable for the generation of "antisense plants".

In summary, it is now clear to researchers working in the area of amino acid transporters, that the heterologous complementation of the double mutant yeast strain 22574d with cDNAs from an *A. thaliana* library was the basis for, and allowed the isolation of, the first plant amino acid transporter, AAP2, as described in the instant application. The methods taught in the application for doing so can be, and have been applied to other model plant systems as well, demonstrating that they are effective in identifying amino acid transporters, and indeed other membrane transport proteins, in a variety of species.

4. I further declare that all statements made herein, of my own knowledge, are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11.2.04

Marion Kwart

Dr. Marion Kwart